BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOME *TRICHODERMA* ISOLATES WITH HIGHLY ANTIFUNGAL ACTIVITY

By

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B.Sc. Agric. Sci. (Agronomy), Fac. Agric., Cairo Univ., 2000
M. Sc. Agric. Sci. (Microbiology), Fac. Agric., Cairo Univ., 2010

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Title of Thesis: Biological and Molecular Characterization of Some *Trichoderma* Isolates with Highly Antifungal Activity
Supervisors: Dr. Mohamed Zakaria Sedik
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Department: Agricultural Microbiology **Approval:**26 /9 /2018

ABSTRACT

Phytopathogen fungi are posing serious problems to the worldwide cultivation systems of economically important plants. Eco-friendly bio-control agents become necessary to help in resolving this problem. In this study, soil and rhizosphere samples were collected from five locations in Egypt. While forty three *Trichoderma* isolates were successfully isolated. Of these forty three fungi, fourteen isolates showed antifungal activity, six of them were belonged to *Trichoderma harzianum*. The other eight isolates included T. *asperellum*, *T. viride*, *T. hamatum* and *T. lixii*. The effect of identified isolates of *Trichoderma* spp. on radial growth and the percentage of inhibition toward the different fungal pathogens were studied in dual culture on Petri dishes. The molecular identification of the isolated *Trichoderma* spp. was done using two taxon-selective primers (ITS1 & ITS4) for the internal transcribed spacer (5.8S-ITS) region in the nuclear ribosomal repeat unit. The sequence BLAST analysis of the ITS sequences showed that the isolates were belonging to *Trichoderma* spp. The phylogenetic relationship was conducted between isolated sequences. Full length of chitinase gene (1.2 Kb) was isolated from two different species and comparatively analyzed between other isolates of high antifungal activity. Select the best medium in terms of growth spore production and biomass yield for the production of *Trichoderma* spp.

Keywords: *Trichoderma*, biocontrol agents, internal transcribed spacer (ITS), chitinase gene, antifungal activity.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my father spirit, to my mother, sisters and my lovely kids (Mohamed and Aya) for support and encouragement they continually offered along the period of my graduation.

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At first, I would like to thank ***ALLAH*** *that allowing me to achieve this work, without his bless any great effort is invaluable.*

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INTRODUCTION

One of the ultimate aims of agricultural biotechnology is to feed an expanding world population. At present, world is facing the twin problem of increasing population and decreasing available land for agriculture. The world population has increased by 90% in the past 40 years while food production has increased by only 25% per head. With an additional 1.5 billion mouths to feed by 2020, farmers worldwide will have to produce 39% more grain (Anonymous, 2000).

In agriculture, worldwide, parasites and pathogens of plants are threat to crop production (Sarah and Paul, 2005). An estimated 37% crop loss is due to pests, of which 12% is due to pathogens. The extensive use of fungicides in various parts of the world for years has increased the pollution level in soil and water, and adverse effect on food quality and human health. Apart from this, the chemicals tend to become less efficient due to the development of resistance among the pathogens over time. Hence, it is necessary to look for alternative disease management practices, which include the use of eco-friendly biological control agents and pathogen-resistant crop cultivars.

Biological control means control of disease through some biological agency that is any living microorganism. Biocontrol may be defined as any condition or practice where by survival or activity of pathogen is reduced through the agency of any other living organism with the result that there is reduction in the incidence of disease caused by the pathogen. Biocontrol of soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of

Trichoderma, *Aspergillus* and *Trichothecium*. There are some antagonistic bacteria like *Bacillus subtilis*, *Enterobacter aerogenes*, *Pseudomonas fluorescence* and *Streptomyces* spp. in disease control.

The specific need of complete disease control, which the biological agent seldom offers due to the problems associated with distribution of pathogen propagates in soil. There is a growing concern in recent years, both in developed and developing countries, about the use of hazardous fungicides for controlling plant diseases. Chemical pesticides have already been proven to cause adverse environmental effects and result in health hazards to human as well as other organisms including beneficial natural enemies. So there is need to develop safer and environmentally feasible control alternatives. Biological control, *i.e.*, the use of biological processes to lower inoculum density of the pathogen in order to reduce the disease producing activities thereby reducing crop loss, is a potential nonhazardous alternative (Chet, 1990).

Among different biological control agents, *Trichoderma* spp. has proved effective and selective enough against most of the fungal diseases. *Trichoderma* spp. has evolved numerous mechanisms that are involved in attacking other fungi. These mechanisms include competition for space and nutrients (Elad *et al.,* 1999), mycoparasitism (Haran *et al.,* 1996 and Lorito *et al.,* 1996), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), inactivation of the pathogen's enzymes and induced resistance (Kapulnik and Chet, 2000). Today, more than 50 different *Trichoderma*-based agriculture products can be found as registered in many countries in five continents, and are sold and applied to protect and improve yield of

vegetables, ornamentals and fruit trees. *Trichoderma* is completely safe and in 55 years of research there has never been a recorded adverse reaction on humans and livestock (Lorito, 2005).

As the skeleton of the fungal cell wall mainly contains chitin, glucan and proteins where mycoparasitism and enzymes that hydrolyze these components are one of the main mechanisms accounting for showing antagonistic activity against plant pathogenic fungi. Chitinase, β-1, 3-glucanase and cellulase are important in the hyper-parasitic mechanism. The distribution of chitinases in nature is very common. It has been established that the chitinase producing *Trichoderma* spp. can be effective biocontrol agents against fungal pathogens (Kubicek *et al.*, 2001).

The ultimate goal of this study was isolation, purification and characterization of some local *Trichoderma* isolates conferring antifungal activity conducted to investigate and characterize some environemently friendly alternatives to protect local crops from fungal pathogenes and, to a lesser extent, fungal pests.

The aims of this work were:

1. Isolation and screening of *Trichoderma* isolates from different soil samples.

2. Screening the effects of *Trichoderma* isolates with high activity against selected important fungal pests.

3. Identification of *Trichoderma* isolates on the basis of morphological features, pathogenecity test and biochemical characteristics.

4. Identification *Trichoderma* isolates using ITS (Internal transcribed spacer) gene, Cloning and nucleotide sequence analysis of ITS gene.

5. Design of oligonucleotide sequences of specific primers to isolate of antifungal gene based on highly conserved domains of different antifungal gene available in nucleotide sequence database.

6. Cloning and nucleotide sequence analysis of antifungal coding sequence.

7. Study the chitinase activities of local *Trichoderma* isolates.

8. Finding the best solutions for economic production of the *Trichoderma* biomass.